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Tissue differentiation in an *in vivo* bioreactor: *in silico* investigations of scaffold stiffness.

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Abstract

Scaffold design remains a main challenge in tissue engineering due to the large number of requirements that need to be met in order to create functional tissues *in vivo*. Computer simulations of tissue differentiation within scaffolds could serve as a powerful tool in elucidating the design requirements for scaffolds in tissue engineering. In this study, a lattice-based model of a 3D porous scaffold construct derived from microCT and a mechano-biological simulation of a bone chamber experiment were combined to investigate the effect of scaffold stiffness on tissue differentiation inside the chamber. The results indicate that higher scaffold stiffness, holding pore structure constant, enhances bone formation. This study suggests that a lattice approach is very suitable for modelling scaffolds in mechano-biological simulations, since it can accurately represent the micro-porous geometries of scaffolds in a 3D environment and reduce computational costs at the same time.

Introduction

In tissue engineering, scaffolds play a critical role in regulating cell activities. They provide a volume in which vascularisation and stem cell differentiation can occur [1]. By virtue of its stiffness and pore structure, the scaffold determines the mechanical environment and thereby modulates tissue differentiation. It is therefore critical to match scaffold mechanical properties to the graft environment not only to prevent mechanical failure of the scaffold but also to create an appropriate mechanical environment for the desired tissue differentiation pathway.

Biomaterials science has mainly used a trial-and-error approach to scaffold design [2]. In order to achieve improved cellular infiltration and better control of the mechanical conditions inside the constructs bioreactors have been designed [2, 3]. Although they have improved the reliability of *in vitro* scaffold experiments, it is essential that scaffolds perform in an *in vivo* environment to promote cellular infiltration and desired tissue formation. Stepstowards this goal could be guided by the use of computational analysis.

Current computational models for predicting tissue formation adopt mechano-regulation theories that link mechanical forces to mesenchymal differentiation pathways. One of the earliest mechano-regulation theories was proposed by Pauwels [4]. He suggested that shear stress and hydrostatic pressure regulates tissue differentiation in fracture calluses. Later, Carter et al. [5] proposed an osteogenic index, described as a combination of distortional strain and hydrostatic pressure, to modulate the process of skeletal tissue differentiation. A more quantitative model was put forward by Claes and Heigele [6] who determined tissue formation by proposing different thresholds of hydrostatic pressure and local stress for bone, cartilage and fibrous tissue differentiation. By characterising the skeletal tissues as biphasic materials, Prendergast et al. [7] proposed that a biophysical stimulus (a combination of fluid flow and shear strain) regulated tissue formation. Although the mechano-regulation theories of Carter et al. [5], Claes and Heigele [6], and Prendergast et al. [7] have been able to capture the main aspects of tissue differentiation, the predictions by the theory of Prendergast et al. [7] have been most successfully correlated with experimental results [8-11]. Another key factor that regulates tissue differentiation is the formation of blood vessels. Newly formed blood vessels provide cells with oxygen and nutrients which are essential for cell proliferation and survival. Since the diffusion of oxygen is limited to a few hundred micrometers from the capillaries, the vascular morphology at the site may play a significant role in determining tissue differentiation patterns [12]. Previous tissue differentiation simulations adopting the mechano-regulation theory of Prendergast et al. [7] within a scaffold have been conducted; the effects of porosity, permeability, Young's modulus, dissolution rate [13] and angiogenesis [14] on tissue differentiation patterns were investigated. However, these studies used a conceptual graft environment/experiment – one of the aims of this paper is to report on how we have extended that work to deal with real scaffolds and their performance in existing mechano-regulated *in vivo* experiments.

Simulations of tissue differentiation inside a mechanically loaded *in vivo* bone chamber have achieved qualitative corroboration in previous studies [10, 11] and shown that the well defined and mechanically controlled environment make bone chambers very suitable for tissue differentiation experiments and simulations. In this study, a bone chamber developed by Tägil and Aspenberg [15] was used (Fig. 1a); consisting of a hollow cylinder with two ingrowth openings at the bottom (Fig. 1b). This bone chamber allows the application of known pressure loads on a defined loading regime, which makes it appropriate for tissue differentiation simulations, particularly for investigating and evaluating the mechanical properties of a scaffold construct.

The objective of this work was to examine the effect of the mechanical properties (Young's modulus) of a scaffold with a known 3D geometry and material properties, on the tissue differentiation process inside a bone chamber, using a computer model. Specifically, we hypothesise that the scaffold can be represented using a lattice model, and that the complexity of the scaffold can thereby be included in the simulation. If this hypothesis can be confirmed then this presents a methodological approach for investigating the interrelationship between scaffold geometry and mechano-regulation in tissue engineering.

Methods

A finite element model of the bone chamber was created (Fig. 1c) to determine the local mechanical environment acting on the cells. Each element was divided into 1000 lattice points (distance between lattice points 10 μm) (Fig. 1d) where each point represented a position a cell and its extracellular matrix [16] or scaffold material could occupy. The cells were allowed to migrate, proliferate, apoptose, differentiate and synthesise new extracellular matrices while new capillaries invaded the chamber,

depending on the surrounding mechanical environment. The scaffold material was included in the chamber by superimposing cross sections (pixels) of processed μCT scans of a highly porous (>90%) collagen GAG-scaffold, which has been used in bone tissue engineering [17-20], on the lattice points (Fig. 1e). The scaffold construct was structured with pore size between 300 and 400 microns. Material data, for both tissues and scaffold, was modelled according to the literature [11, 21].

A random walk algorithm [16] was implemented for cell migration. Cell proliferation (mitosis) occurred by allowing mother and daughter cells to randomly occupy neighbouring free lattice points in 21 different states [22].

Cell differentiation also occurred in a random fashion but modulated by the site vascularity [12] and a biophysical stimulus described as a combination of fluid flow and shear strain [7]. Different levels of stimulus determined the differentiation of mesenchymal stem cells into osteoblasts, chondrocytes and fibroblasts which formed bone, cartilage and fibrous tissue, respectively. High levels of biophysical stimulus promoted differentiation of fibroblasts, whilst intermediate stimulus resulted in chondrocytes. Low levels of mechanical stimulation favoured osteoblastic differentiation but only in well vascularised areas where oxygen tension was high. Mesenchymal stem cells (MSCs) in regions under a mechanical stimulus favorable for osteoblast differentiation but with poor vascularity followed the chondrogenic pathway rather than the osteogenic [12].

Capillaries were described as a sequence of endothelial cells. Capillary tips could extend either in the previous direction (persistence), a random direction, or along a concentration gradient (e.g., VEGF that acts as an angiogenic factor and is assumed to be released by hypertrophic chondrocytes) [12]. Each vessel had a possibility to branch with a probability determined by the length of the vessel [12], where longer vessels had a higher probability. The growth of the vessels was restricted by anastomosis (the fusion of two sprouts) and a high mechanical stimulus [12].

The tissue differentiation process inside the bone chamber was modelled as an iterative process where each iteration corresponded to 12 hours. The chamber was initially filled with scaffold material and granulation tissue. As an initial condition, MSCs and endothelial cells were seeded at the ingrowth openings. Next the cells began to infiltrate the chamber by migration and proliferation. After the MSCs reached a maturation age (6 days) they differentiated depending on the biophysical stimulus and the site-specific vascularity, with cell phenotype specific rates [23]. Since the lattice points in an element could not only represent different cell phenotypes which synthesize extracellular matrices with different material properties but also have material properties of the scaffold construct, a rule of mixtures was used to determine the material property of each element [24]. Also, in order to prevent an unphysiological rapid change in material properties, the values were averaged over 10 previous iterations.

Correspondingly with the experiments [15], the chamber was kept unloaded for 3 weeks allowing tissue to grow in and then subjected to 2 MPa loading, every 12 hours, for the 6 following weeks. The tissues and the scaffold inside the chamber were assumed to be under very low loading representing normal rat blood pressure during the unloaded time period and were subjected to 0.02 MPa [11]. The effect of scaffold Young's modulus on tissue differentiation patterns was investigated by varying the scaffold Young's modulus within experimentally reported values [25-27]: 0.001, 0.01, 0.1, 1.0, 10, 100 and 1000 MPa.

Results

After 9 weeks, for less stiff scaffolds (Young's modulus between 1-1,000 kPa) simulations predicted a large amount of fibrous tissue formation due to high fluid flows and shear strains inside the chamber and small amounts of chondrogenic and osteogenic differentiation (Table 1). By increasing the stiffness of the scaffold material, larger amounts of chondrocytes and osteoblasts were predicted and a decrease in fibroblast differentiation was observed, see Table 1 and Fig. 2. In the simulation where a rather stiff scaffold was considered (E=1,000 MPa), a thick layer of chondrocytes surrounded by osteoblasts with no fibroblasts was predicted (Fig. 2). The results also showed a greater and higher endothelial cell invasion in the chamber due to the lower mechanical stimulus environment induced by stiffer scaffold material (a population increase from 6.6% to 8.2% of the chamber volume for E=0.001 – 1000 MPa). However, this has no significant effect on the differentiation outcome since the high porosity of the scaffold provides easy access for the formation of the new capillaries in the entire chamber for all simulated scaffold Young's modulus. Therefore the predictions clearly indicate a crucial role for scaffold stiffness in bone regeneration.

Discussion

This study shows that the lattice modelling approach has the benefit of being able to accurately include the micro-porous geometries of scaffolds in mechano-biological simulations that have previously been done using smaller mesh size [28]. The lattice points are successful in representing cells and their extracellular matrices as well as scaffold material and the high density of lattice points allows for explicit modelling of scaffold pore configurations that are otherwise difficult to capture in standard FE-models. The results obtained in this study indicate that the large pore size allows cells and blood vessels to easily enter and fill the scaffold and to supply sufficient nutrients and oxygen. The scaffold construct is very porous and only 10% of the chamber is filled with scaffold material. The simulations show that increasing scaffold mechanical stiffness from 1 to 1000 kPa does not have a significant effect on the tissue differentiation outcome after 9 weeks whereas a higher mechanical stiffness ($E > 1$ MPa) enhances bone differentiation in highly porous constructs. This suggests a loading-specific scaffold stiffness threshold above which bone regeneration is facilitated.

Simulations of scaffold stiffness being ≥ 10 MPa showed a significant increase of bone and cartilage formation. A large amount of fibrous tissue was however still predicted at the bottom of the chamber in the vicinity of the ingrowth openings, where the fluid flows and shear strains are high. When implementing $E = 1$ GPa, fibrous tissue was no longer found at the ingrowth openings and only bone and cartilage could be observed in the chamber. A further increase in scaffold stiffness ($E = 2$ GPa) concluded in very low biophysical stimulus and only bone differentiation (results not shown).

Although we have been careful to assign values to parameters consistent with literature [23] it is a limitation of the model that some parameters are difficult to define precisely, such as the permeability of different tissues, maturation age and cell process rates. The scaffold construct was not considered to be degradable which could be essential to the process of tissue differentiation. Moreover, the plastic deformation (buckling) of the scaffold struts was not taken into consideration. This might have a major effect on the cell access to the interior of the scaffold by clogging the openings. Although the simulations are non-deterministic in that they adopt stochastic modelling of cell processes, a previous study has shown that this cannot capture the inter-specimen variability found in experiments of the bone chamber [11]. Hence it must be noted that the simulations performed in this study are expected to be subjected to a larger variability in an *in vivo* animal population.

Despite the above limitations, model predictions were able to capture the effect of scaffold stiffness on tissue differentiation patterns which corroborate with experimental observations [29, 30] In contrast to previous models, this framework adopts not only a mechanistic approach but also considers the effect of angiogenesis on tissue differentiation patterns in a well controlled environment. It simulates an already existing *in vivo* experiment that has been successful in showing the significant effect of mechanical loading on the tissue differentiation process.

We conclude that our hypothesis is confirmed; that the lattice modelling approach is suitable for studies of tissue differentiation inside scaffolds, as this technique was not only able to represent cellular processes explicitly but captured also the complex micro-porous geometry of the scaffold. With the lattice model a smaller mesh size is not necessary for capturing the porous configuration of a scaffold which in turn reduces computational time and problems related to the meshing of the scaffold. The result of this study reaffirms the importance of the design of tissue engineering scaffolds and suggests a threshold for scaffold stiffness above which osteogenesis is enhanced. The successful implementation of the lattice model, for representing complex scaffold pore geometries, advances a novel approach for tissue engineering scaffolds, specifically for computational investigations of the relationship between scaffold pore geometries and mechano-regulation.

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Figures

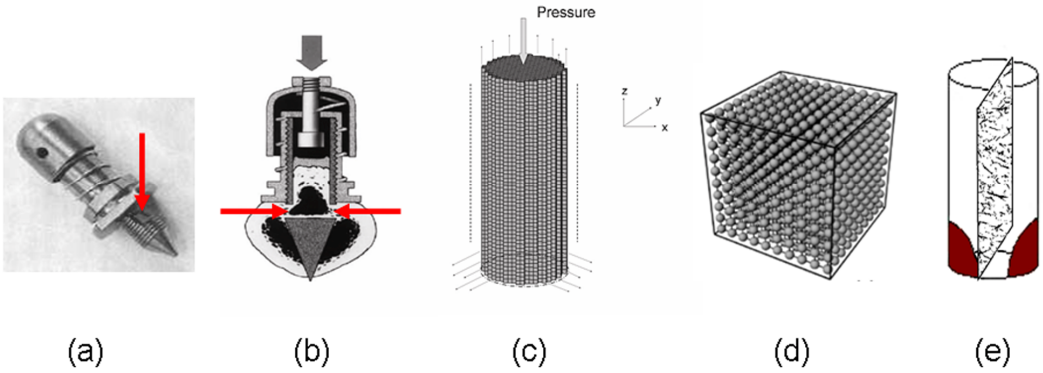

Fig. 1 a) The bone chamber, where the arrow points at an ingrowth opening b) Cross-section of the bone chamber [15] where the thin arrows point at the ingrowth openings and the thick arrow points at the piston by which loading is applied. c) FE-model of the interior of the bone chamber where the chamber wall was modelled as boundary conditions; ↑↑↑↑: free fluid flow; ----: $u_x = u_y = 0$; -·-·-·-: $u_x = u_y = u_z = 0$. d) A finite element containing 10x10x10 lattice points for the simulation of cell activity [13]. e) Sketch of the bone chamber illustrating the scaffold construct at the mid-cross-section of the chamber; 'quadrants' of the openings and the sections through the biomaterial are represented as dark patches in the cross-section

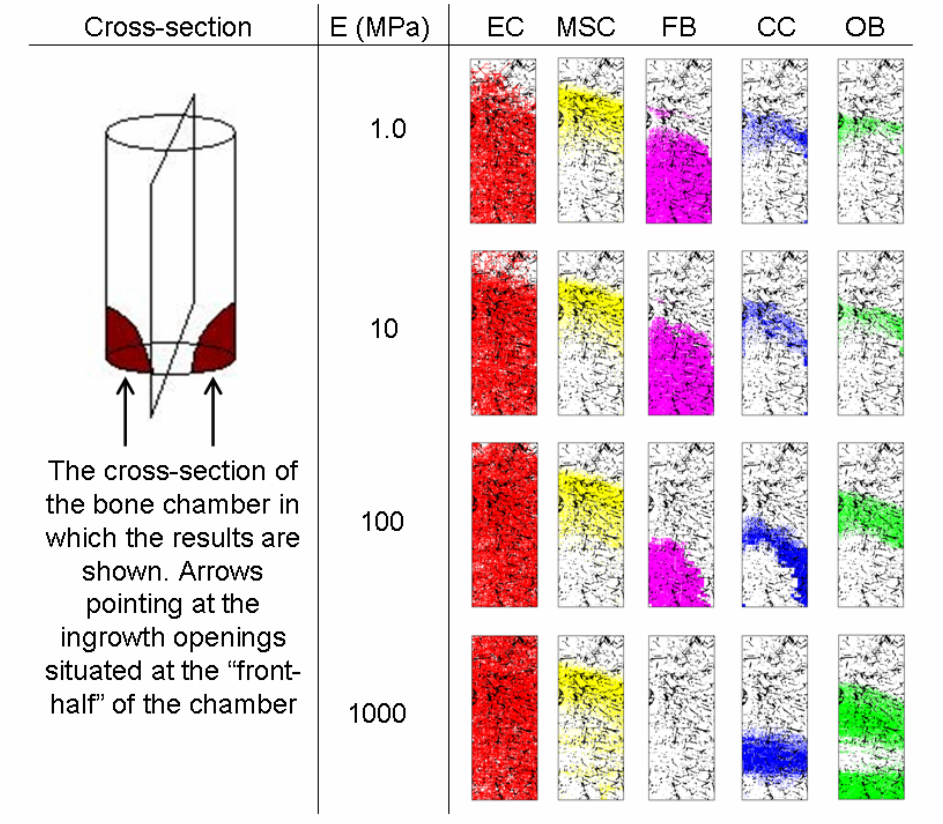

Fig. 2 Mid-cross-sections of the chamber where the coloured lattice points illustrate endothelial cells (EC), mesenchymal stem cells (MSC) fibroblasts (FB), chondrocytes (CC) and osteoblasts (OB). The black lattice points scattered in the entire chamber denote the scaffold occupying the chamber

Tables

Scaffold Young's Modulus (MPa)	Fibroblasts (%)	Chondrocytes (%)	Osteoblasts (%)
0.001	89.5	5.8	4.7
0.01	89.6	5.9	4.5
0.1	89.0	5.7	5.3
1.0	89.2	5.9	4.9
10	82.6	8.7	8.7
100	59.3	19.7	20.1
1000	1.6	32.7	65.7

Table 1 Percentages of the differentiated cell phenotypes inside the bone chamber for different scaffold stiffness